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Article

Factors associated with leucism in the common blackbird *Turdus merula*

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Leucism is the total or partial lack of melanins in the skin and associated structures (i.e. hair or feathers). Little is known about the factors influencing this chromatic aberration although some local studies suggest that there is an effect of habitat, age and sex. To test these hypotheses and expand our knowledge on leucism, we carried out a large-scale study using common blackbirds *Turdus merula* as our model species. Given the poor information available on this topic and the variability of methodological approaches, we used three different methods to assess the effect of these variables in the presence of leucism: transects, bird captures and citizen science information (pictures from internet). We found an effect of habitat indicating that there are more leucistic blackbirds in cities than in non-urban areas. In addition, we found a positive association between presence of white feathers and age providing the first large-scale support for the progressive graying hypothesis in birds. This chromatic aberration was also influenced by sex, with males showing higher probability of leucism than females, although only for the capture data, indicating that the method used to study this phenomenon can partly influence our conclusions, and therefore suggesting caution when planning future studies in this topic.

Keywords: avian coloration, citizen science, urbanization

Introduction

Plumage coloration in birds is mainly the result of the deposition of pigments in the feathers (Fox and Ververs 1960). The most important pigments in bird coloration are carotenoids (red and yellow coloration) and melanins (brown and black coloration) (McGraw 2006a, b). Disturbances in the deposition of pigments produce chromatic aberrations (van Grouw 2013). The most common of these aberrations, mostly associated with melanin-based pigmentation, is leucism or partial albinism (Guay et al. 2012), the total or partial lack of melanins in the feathers. Leucism is



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characterized by having white patches (in the most common cases only in the feathers) but normal coloration in some parts of the body (i.e. have normal-colored eyes, bill, skin and feet) (Guay et al. 2012).

Despite our detailed knowledge on the origin of this chromatic aberration, the ecological factors that affect it have been poorly studied. Evidence suggests that leucism is associated with certain habitats, for instance, Il'enko (1960 cited in van Grouw 2012) found that leucistic house sparrows *Passer domesticus* were more common in cities (1–2%) than in non-urban areas (about 0%). Holyoak (1974) found similar percentages in other bird species of the London area. Finally, Rollin (1953), reported a higher percentage of leucistic blackbirds in the city than in nearby rural areas. However, these studies took place at a local level (a single city), which makes it impossible to determine whether these findings are due to local or habitat effects, and therefore preventing generalization. Moreover, the ecological factors that trigger leucism are not entirely clear (Guay et al. 2012) but, given the possible relationship with urban habitats, it is possible that they are environmentally associated factors.

According to van Grouw (2013), leucism can be explained by three different, not mutually exclusive, mechanisms: nutritional alterations, heritable mutation and 'progressive greying'. Nutritional alterations, like a poor diet, may be caused by external factors, that can produce alterations in the pigmentation. This has been tested mainly in carotenoid-based pigments (Isaksson and Andersson 2007), but can also occur in melanin-based pigments (Rollin 1959) because tyrosine is necessary for the synthesis of melanin, and low levels of this amino acid can produce greying (van Grouw 2013). This mechanism could explain the association between leucism and cities (Rollin 1953) that have been suggested to be poor nutritional habitats (Isaksson and Andersson 2007, Møller et al. 2010). This link may also explain carotenoid-associated color changes in urban birds (Giraudeau et al. 2015).

The mechanism of genetic mutation proposes that leucism is a inherited disorder. Lack of melanin in feathers is caused by issues in the migration of melanoblasts (cells that are transformed into pigment cells) during the embryonic development (van Grouw 2013). The genetic basis of this phenotypical change has been associated with more than ten mutations in the feral pigeon *Columba domestica* (van Grouw and de Jong 2009 cited in van Grouw 2013). Although this possible cause of leucism is mainly due to intrinsic genetic factors of the individual, it could also have an extrinsic environmental component. There are more leucistic birds in areas with high levels of mutagens (Ellegreen et al. 1997), therefore rates of leucism may be greater in the cities because of the elevated levels of mutagens (Yauk and Quinn 1996).

Progressive greying involves the continuous loss of pigment cells with age (van Grouw 2013). By this mechanism the number of white feathers would increase after every moult, similarly to what happens in humans or other mammals (Henner et al. 2002, Westgate et al. 2013). The 'free radical theory of aging' states that tissues and cells suffer a progressive

accumulation of reactive oxygen species with age that, in the case of humans, produces a progressive loss of melanocytes and the loss of pigmentation in the hair (Arck et al. 2006). Furthermore, antioxidant levels have been associated with melanogenesis in other mammals and birds (Galván and Alonso-Alvarez 2009, Galván et al. 2012). Therefore, the higher incidence of leucistic birds in cities could be explained by more older birds living in urban than in non-urban areas, as seems to be case (Ibáñez-Álamo et al. 2018), or even due to individuals living in cities suffering a higher oxidative stress than natural areas (Hutton and McGraw 2016).

Coloration is a vital component of individual survival and reproduction (Hill and McGraw 2006). Leucism therefore likely entails a significant cost and decreases an individual's fitness (Dyck 1985). Leucism will likely increase detection by predators (i.e. reduced camouflage) (Ellegreen et al. 1997), and alter success in mate choice (i.e. melanin-based signals) (Møller and Mousseau 2003). Because of this potential negative effect in mate choice, several studies have tried to test whether leucism is sex-biased, but there is no consensus. Some studies suggest females are more prone to be leucistic (Il'enko 1960 cited in van Grouw 2012), but others found no differences between sexes (Slagsvold et al. 1988, Møller and Mousseau 2001). Additional studies in different geographical areas or with different species will provide an interesting addition to this debate.

Leucism in birds has been studied with different methodologies. Most commonly, leucism prevalence is reported for captured individuals (Il'enko 1960 cited in van Grouw 2012, Slagsvold et al. 1988, Ellegreen et al. 1997, Møller and Mousseau 2001), including museum specimens (van Grouw 2013). Observations of individuals in the field has also been used (Rollin 1953, Holyoak 1974). More recently, chromatic aberration studies have profited from new technologies that allow the use of citizen science data (i.e. bird images available on the internet) (Husby 2017). To our knowledge, there is no comparison on whether the different methodologies available provide similar and consistent results regarding chromatic aberrations, or leucism in particular. This issue is important if we want to advance in our understanding on this topic and extract general conclusions from past and future studies.

We had four main aims in our study: First, we used meta-replicated field data to test if leucism is associated with urban habitats. Second, we investigated whether there are sex differences in leucism. Third, we analyze the progressive graying hypothesis by testing for an association between leucism and age. Finally, we compared the results obtained from leucism estimates derived from three different methods (bird captures, transects and citizen science data). We used all three methodological procedures to quantify the presence of leucism in urban and non-urban adult blackbirds.

We used the common blackbird *Turdus merula* as our model species since it has a wide geographical distribution (Collar 2005) and can inhabit both urban and non-urban areas (Evans et al. 2010, Ibáñez-Álamo and Soler 2010, Møller et al. 2014). Its plumage coloration is based only on melanins (van Grouw 2006, 2013) facilitating the

interpretation of white patches in their plumage and sexes can be easily recognized in the field. We predict that cities will contain a greater number of leucistic individuals in comparison with non-urban habitats and a higher prevalence of leucism in old birds. Based on previous studies (Slagsvold et al. 1988, Møller and Mousseau 2001), we expect no sex differences in leucism. We also predict that quantifying leucism with the bird in hand (captures) rather than in the distance (transects or citizen science data) will provide more accurate information on this phenotypic trait.

Material and methods

Transects

During the breeding season (March–April) of 2017, we observed 20 blackbirds in each of 10 Spanish blackbird populations. Five populations were in urban areas and five in non-urban areas geographically paired (Fig. 1). The average distance (\pm SE) between the paired urban and non-urban areas was 33.3 ± 7.7 km, which is an order of magnitude greater than the mean natal dispersal of blackbirds (Paradis et al. 1998). Urban areas consisted of parks and gardens always located in the core area of the cities. Using satellite images (i.e. Google Earth), we calculated the percentage of land-built area in a 1 km² square centered in the observation points,

which confirms that the selected areas matched with the urban/suburban categories (> 30% built area) described by Marzluff et al. (2001). Non-urban areas were located mainly in forested or agricultural areas with minimum human perturbation and matched with the rural/wildland categories (< 20 % built area) from Marzluff's classification.

These observations were carried out by the same researcher and while doing linear transects of variable length (2 to 8 km), depending on blackbird density at each studied population. To avoid counting the same individual twice all transects were visited in a unique occasion. The time lag between sampling paired urban and non-urban areas was less than a week to prevent differences in detectability of individuals associated with variation in breeding stages. Based on information provided by local ornithologists, we also standardized our observations among locations to the same breeding stage (i.e. nestling period of the 1st brood). These transects were carried out using a GPS device guaranteeing not going through the same area more than once and avoiding noting individuals that were behind us. Each blackbird encountered and fully visible (i.e. not partially hidden) was visually inspected with the use of binoculars for at least one minute (otherwise discarded) and assigned as leucistic when they presented at least one white feather. Each blackbird was sexed based on plumage coloration (Svensson 1992). Due to the distance of observation of many of these individuals, it was not possible to attribute the age.

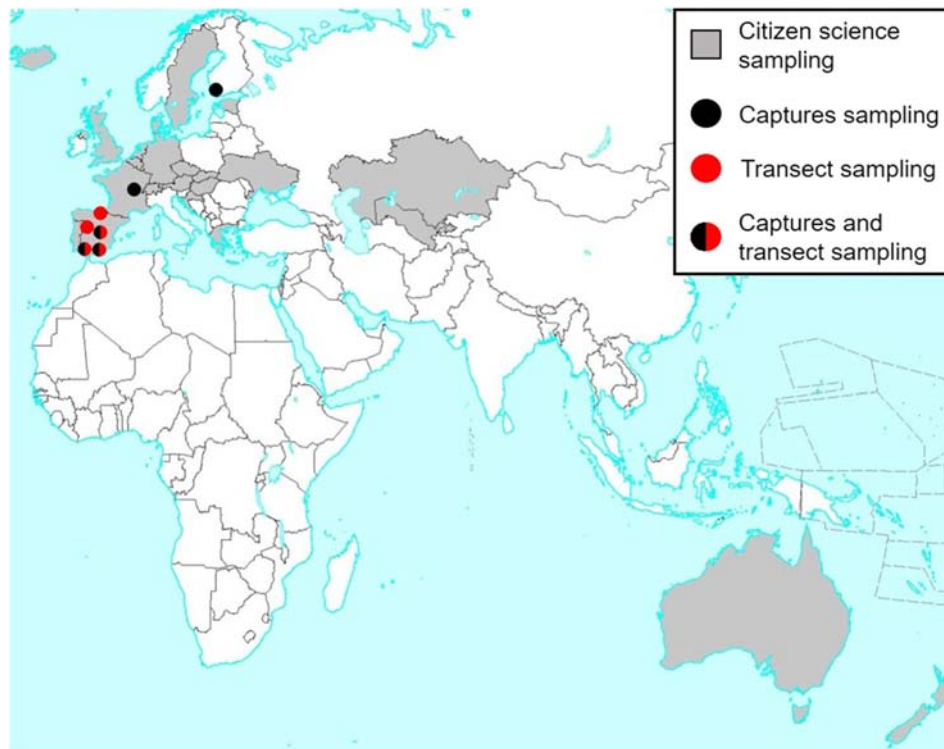


Figure 1. Map showing the countries with blackbirds for the citizen science sampling (painted in grey) and the locations of transects (red dots) and captures (black dots). Locations in which both captures and transects were carried out are shown by a red-black dot. For each dot in the map, an urban and a non-urban population of blackbirds were sampled.

Bird captures

During the breeding season (March–July) of 2015, we captured 25 ± 2.4 blackbirds from each of 10 paired European populations (5 cities and 5 forests) spread from southern to northern Europe (Fig. 1). The average distance (\pm SE) between urban and non-urban paired areas was 29.8 ± 3.8 km. Similar to the transect method, the urban habitat consisted of parks and gardens and the non-urban habitat was located in protected forested areas with minimal human activity. As in the transect methodology, this habitat classification matched with the urban/suburban and rural/wildland categories described by Marzluff et al. (2001). See Ibáñez-Álamo et al. (2018) for additional information on the sampling locations and confirmation of habitat differences according to another standard urbanization index.

All birds were captured using mist nets and were ringed with coded metal rings. Age (1 yr old or ≥ 2 yr old) and sex was determined according to Svensson (1992). All individuals were checked for the presence of any leucistic feathers before release.

Citizen science data

We collected pictures available on the internet by using a web application called Morphic, developed and tested as a tool to study geographic color patterns in animals (Leighton et al. 2016). This application allows users to search for photographs from Google Images employing specific keywords ('*Turdus merula*', 'common blackbird' and 'Eurasian blackbird' in our case) and ensures no duplicates are retrieved. The image search was conducted on the 14 May 2017 and provided 1200 pictures. For each photographed blackbird we collected the following variables: age, sex, presence/absence of leucism, locality and habitat type. Only those pictures providing information from all variables were used in our analyses (186 pictures from three different continents; Fig. 1). Sex and age was determined as in the previous methodologies. To assess the error rate of age and sex determination using internet photographs, two independent observers ranked age and sex from several pictures ($n = 37$) and obtained a repeatability of 86 and 100% respectively. Locality indicated the small scale geographical area (e.g. province) where the picture was taken and was based on the UTM coordinates associated to the photograph. We used a similar method for assigning the locality of transect and capture sites in order to use a standardized geographical variable. Finally, habitat type estimation was done using also a similar procedure as that previously described. Based on a 1 km² square centered at the point where the photograph was taken, we assigned each individual to either urban or non-urban habitats, which correspond with the urban/suburban or rural/wildland categories proposed by Marzluff et al. (2001).

Statistical analysis

We first performed a GLMM (generalized linear mixed model, binomial distribution and logit link function) with all collected data (637 individuals; Supplementary material Appendix 1 Table A1) to investigate the effects of sex, habitat and the different methodological approximations on the presence of leucism in an individual. Leucism (presence/absence) was tested as the response variable, with several fixed factors (type of the methodology, habitat and sex) included. We also included the interactions between methodology and sex or habitat to explore potential biases associated with the different methods. We included locality as a random factor to account for samples from the same geographic site. Second, to analyze the specific effect of age on the probability of leucism, we fitted a binomial GLMM (logit link function) including only the captures and citizen science data (only data with age information available; 437 individuals). The model was the same as described above but adding age as an additional fixed factor and their interaction with methodology. We also added the interaction between age and habitat because of the known effect of urbanization on the age structure of blackbirds (Evans et al. 2009, Ibáñez-Álamo et al. 2018). Finally, we run two sensitivity analyses to explore whether the different geographic scales of each methodology could explain our results. We did so by running the previously described models using only the data from Europe (i.e. we restricted the data obtained using the citizen science methodology to European blackbirds).

All models were run using the 'lme4' package (ver. 1.1-13; Bates et al. 2015). We followed a stepwise model selection procedure to obtain a minimum model from the initial starting model. The factor with the highest p-value according to a likelihood ratio tests (LRT) for nested models was removed subsequently until all included terms were significant. We used the 'lmerTest' package for the LRT (ver. 0.9-35; Zeileis and Hothorn 2002). The main effects were tested with Wald tests using the 'car' package (ver. 2.1-5; Fox and Weisberg 2011). Tukey post hoc analysis was performed with the 'phia' package (ver. 0.2-1; De Rosario-Martinez 2015). All analyses were performed with the R software (ver. 3.4.1, R Core Team).

Data deposition

Data available from the Dryad Digital Repository: <<http://dx.doi.org/10.5061/dryad.jr89m60>> (Izquierdo et al. 2018).

Results

The overall percentage of leucistic individuals in each habitat estimated by each methodology already shows some differences suggesting a higher prevalence of leucism in cities (Table 1). This habitat effect is confirmed with the first statistical analysis that includes all data and shows that leucism is significantly associated with the type of habitat (estimate \pm SE: 0.97 ± 0.28 ; Table 2). Individuals in urban

Table 1. Percentage and frequencies of leucistic individuals in the different habitats for the different methodologies.

Sampling	Urban		Non urban	
	Percentage	Frequencies	Percentage	Frequencies
Google images	19.6	22/112	9.5	7/74
Captures	18.6	24/129	11.5	14/122
Transect	10	10/100	0	0/100

habitats were more likely to show leucism than those in non-urban habitats (Fig. 2). In addition, we found a significant interaction between methodology and sex (Table 2) indicating that males were more likely than females to be leucistic according to the captures (Tukey post hoc: $\chi^2 = 15.12$; $df = 1$; $p = 3.02e-04$; estimate \pm SE: 2.18 ± 0.55) but not with other methods (Tukey post hoc: $p = 0.51$ and 0.96 for transects and citizen science respectively; Fig. 3).

In the second analysis, using only captures and citizen science data, we found a higher proportion of leucistic birds among older individuals (estimate \pm SE: 0.81 ± 0.36 , Wald test: $\chi^2 = 5.06$; $df = 1$; $p = 0.02$; Supplementary material Appendix 1 Table A2). This model also showed a similar pattern to that described above for the interaction between methodology and sex (Supplementary material Appendix 1 Table A2), and for habitat, although in this case it was marginally significant ($p = 0.06$) probably due to the smaller sample size. There was no significant effect of any other interaction (Supplementary material Appendix 1 Table A2).

Discussion

Our study provides the first large-scale evidence of the environmental and individual factors that are associated with high rates of prevalence of leucism in a bird species. We found that cities hold a larger proportion of leucistic individuals in general (Fig. 2), suggesting that this common chromatic aberration is associated with human-induced habitats, and potentially influenced by anthropogenic causes. Our findings fit with those of previous local studies that reported a similar effect of urbanization in other species (Rollin 1953, Il'enko

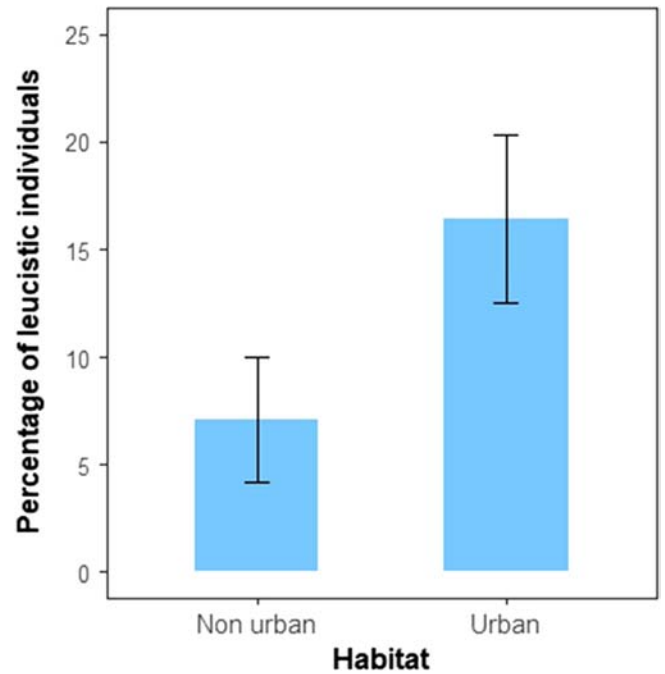


Figure 2. Percentage of leucistic individuals according to habitat for all methodologies combined (95% confidence interval).

1960 cited in van Grouw 2012, Holyoak 1974). Although, they did not support their observed differences statistically, we used Rollin and Holyoak's data to run simple statistics and found a significant effect for house sparrows and common starlings *Sturnus vulgaris* but not for woodpigeons *Columba palumbus* (Supplementary material Appendix 1 Table A3). The results for the blackbirds were contrasting as leucism was significantly associated to urban habitats based on Rollin's data but not on Holyoak's study (Supplementary material Appendix 1 Table A3). The prevalence of leucism in urban habitats provided in these articles (range: 0.3 to 9.5%) are considerably lower than those shown in our study (Table 1), particularly those obtained using the captures or citizen science methods. Some of these previous studies used non-captured individuals (Rollin 1953, Holyoak 1974),

Table 2. Minimum model terms, rejected terms and random terms of the binomial GLMM general model (including transect, capture and citizen science data) explaining the presence/absence of leucism. Sample size: 637 individuals. p-values marked in bold indicate significance ($p < 0.05$). Likelihood ratio tests (LRT in table) were calculated for the random effect.

	Estimate	SE	X ²	df	p
Intercept	-3.940	0.586			
Habitat (urban)	0.973	0.282	11.892	1	0.001
Method (citizen science)	1.564	0.677	10.596	2	0.005
Method (transect)	-0.263	0.902			
Sex (male)	2.148	0.552	7.385	1	0.007
Method (captures): sex (male)	2.177	0.551	9.004	2	0.011
Rejected terms					
Method (citizen science): habitat (urban)	0.281	0.610	0.256	2	0.80
Method (transect): habitat (urban)	16.914	81.467			
Random term					
Locality		LTR	3.405		0.065

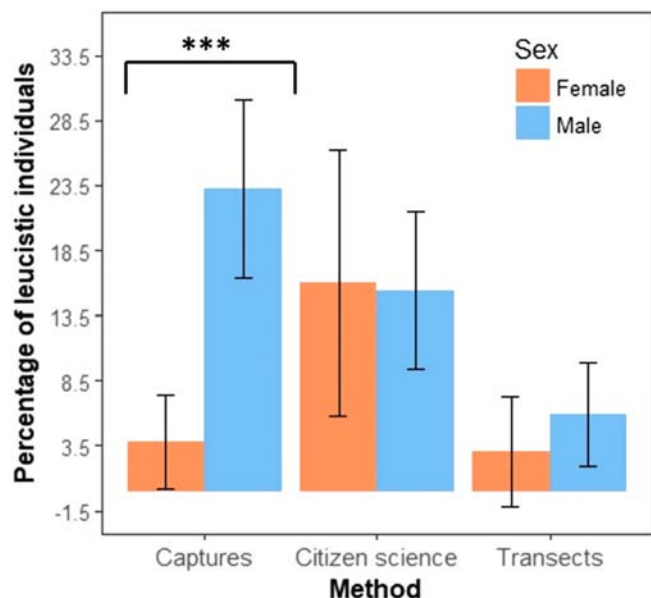


Figure 3. Percentage of leucistic blackbirds by sex for each of the methodological procedures used (95% confidence interval). Significant Tukey post hoc analysis are indicated with asterisk.

which could explain these differences and the more similar percentages found for the transect method. In addition, between species differences in leucism prevalence may be the result of their different ecology or intrinsic factors (i.e. physiological or life-history traits). Our results are also in agreement with previous studies that, more generally, indicate that cities could negatively affect the coloration of birds (Eeva et al. 1998, Giraudeau et al. 2015) and provide the first solid evidence for this negative relationship between cities and plumage color in the case of chromatic aberrations.

There could be several explanations for the association between urban habitats and leucism in birds. One possible cause is that blackbirds from urban areas may be subject to a higher level of mutagens that can lead to chromatic aberrations (Ellegreen et al. 1997). Another factor could be that, within these environments, birds are subject to nutrient-poor diets (Isaksson and Andersson 2007), perhaps including a lack of tyrosine. A third possible cause may be that non-urban populations are subject to greater predation pressure than in urban habitats, where there is a reduction of natural predators (Møller and Ibáñez-Álamo 2012). The latter would imply that, although the presence of leucism was originally the same in both habitats, there is a selective disappearance of leucistic (more conspicuous) individuals in the forests and not in cities. Finally, urban habitats have been associated with a strong negative effect in the oxidative balance of animals (Hutton and McGraw 2016) and given that oxidative stress can produce alterations in melanogenesis due to mutations in the process of synthesis (Møller and Mousseau 2001), alterations in proteins (such as melanogenesis enzymes; Surai 2002) or

melanocyte apoptosis (Arck et al. 2006), it can be a likely explanation for our findings too.

Our study also presents the first large-scale support for the progressive graying hypothesis in birds as we found a larger prevalence of leucism in older blackbirds. Interestingly, this effect was independent on the habitat and the methodology, suggesting that it is a general process for blackbirds regardless of the type of habitat and the method. Our results match with the findings of the only other study that investigated this hypothesis before. With a series of experiments using blackbirds, Sage (1962) also found that the number of white feathers increases as blackbirds get older. Future studies should focus on other species and groups of animals, with a particular emphasis in longitudinal studies that explore the relationship between this phenotypic change and the process of ageing. Another interesting future avenue of research is related to the ‘free radical theory of aging’ (Arck et al. 2006) and should explore the potential relationship between senescence, graying and oxidative stress. The link between age and leucism found here, in addition to the evidences showing that the proportion of old blackbirds is higher in the city than in non-urban areas (Evans et al. 2009, Ibáñez-Álamo et al. 2018), could also explain the detected association between urban habitats and this chromatic aberration.

The sexual differences in the incidence of leucism were unexpected as males were the most affected by partial albinism. This effect was only detected for the capture methodology clearly indicating that there might be biases in the studies of leucism depending on the way we collect the data. Our results showed a higher prevalence of leucistic males and contrast markedly with previous studies reporting a higher proportion of leucistic females than in males in house sparrow *Passer domesticus* and tree sparrow *P. montanus* (Il’enko 1960 cited in van Grouw 2012, van Grouw 2012). Additionally, no differences between sexes were reported in the hooded crow *Corvus corone* and the barn swallow *Hirundo rustica* (Slagsvold et al. 1988, Møller and Mousseau 2001). It is likely that the factors determining leucism affects sexes of different species to different degrees, so it is not possible to conclude that there is a general association between leucism and sex in birds. Focusing on the blackbird, we speculate that the most important cost associated to leucism will be related to predation rather than sexual selection given that, in mate choice bill coloration is more important than feather coloration (Faivre et al. 2001). However, no study has investigated the effect of leucism on blackbird’s mate choice and thus we cannot completely rule out this possibility. One possible reason why there may be fewer leucistic females is because there is a stronger selection against them due to predators. It has been demonstrated that there is a bias in adult mortality that favors males, especially during the breeding season (Post and Götmark 2006). This would imply that, regardless of the leucism, there is already a selection against females. In addition, if we add that female blackbirds are more cryptic than males (Collar 2005) and the fact that leucism disrupt the camouflage of

birds, there will be an even stronger selection against these leucistic females than males. Other alternative hypotheses that could explain these sex differences could be that some of the physiological mechanisms determining leucism affect differently each sex. Also, the plumage of male blackbirds is mainly based in the pigment eumelanin, while female plumage contains also pheomelanins (Negro et al. 2018). It has been suggested that in species where plumage coloration involves the production of differential amounts of pheomelanin, the sex with lower levels of this pigment can suffer higher levels of oxidative damage (Negro et al. 2018), which consequently could be more affected by leucism.

We found a clear effect of the methodology used to study leucism and suggest caution to carefully design future studies on the topic. This effect, for example, seems to be very important regarding the study of sex differences, as citizen science data fail to detect sexual differences in prevalence of leucism, but not relevant enough for habitat or age effects. In general, the capture methodology seems to be the best option as it provides additional information not possible (or difficult) to collect with other methodologies (i.e. age information). In addition, captures of birds allow for the inspection of the whole body (an important limitation of the citizen science images) that could be responsible of the differences in the sex effect (Fig. 3). Selective publication might also explain the trend of a higher incidence of leucistic blackbirds found by the citizen science methodology, however, Leighton et al. (2016) concluded that this potential bias did not affected the conclusions of their study using Morphic. An alternative explanation for the differences between methods could be the different geographical scale used for each method. Geographic variation in plumage coloration is important (Bailey 1978, Dale et al. 2015). It is possible that the citizen science data provides a higher incidence of leucistic blackbirds than the other two methods because this incidence is higher outside the studied area in Europe. However, this explanation seems unlikely based on our sensitivity analyses in which we run the same models but using only data from Europe. These additional analyses showed similar results to the ones including information from other continents (Supplementary material Appendix 1 Table A4 and A5) supporting that our findings are robust and pointing to methodological rather than geographical effects. Overall, our findings on the methodological bias support previous results on the topic as the majority of these studies have used captured individuals to investigate leucism (Il'enko 1960 cited in van Grouw 2012, Slagsvold et al. 1988, Ellegren et al. 1997, Møller and Mousseau 2001).

Overall, our study provides important pieces of information to better comprehend the patterns and ecological factors associated with avian leucism. We have demonstrated, for the first time, a general (large-scale) association between urban habitats and leucism, which suggests that this chromatic aberration is probably influenced by environmental factors. We also present one of the first pieces

of evidence supporting the progressive graying hypothesis in birds. In addition, we found that the methodology used could be very important to detect some effects, like the male-biased prevalence of leucism in blackbirds that can only be detected with the inspection of the bird in the hands of the researcher (capture method). Future studies should carefully evaluate the best method for their objectives, and if possible, we recommend the use of captured birds.

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Conflicts of interest – The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Permits – Our study was approved by the Ethical Committee of the Consejo Superior de Investigaciones Científicas (CSIC) with the reference number 258/2015 and complies with all relevant EU legislation. We obtained local and regional permits to capture and extract blood samples from blackbirds in all locations.

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Supplementary material (Appendix JAV-01778 at <www.avianbiology.org/appendix/jav-01778>). Appendix 1.